WEST Search History

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DATE: Wednesday, April 14, 2004

Hide?	Set Name	Query	Hit Count
	DB=PGPB,USPT; PLUR=YES; OP=ADJ		
☐	L12	L11 and L7	19
☐	L11	19970610	51
☐	L10	L9 and (dna or cdna or polynucleotide or nucleotide or nucleic acid)	282
☐	L9	L8 and Pyrococcus furiosus	286
☐	L8	protease or proteinase or peptidease or Endopeptidase or Endoprotease or Endoproteinase	60248
☐	L7	L6 or L5 or L4 or L3 or L2 or L1	28757
☐	L6	(536/23.2)!.ccls.	10680
☐	L5	(435/320.1)!.ccls.	23166
☐	L4	(435/252.3)!.ccls.	8057
☐	L3	(435/219)!.ccls.	918
☐	L2	(435/212)!.ccls.	776
☐	L1	(435/183)!.ccls.	4460

END OF SEARCH HISTORY

h e b b cg b chh e h f e cf

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=> d full his

     (FILE 'HOME' ENTERED AT 13:49:22 ON 14 APR 2004)

     FILE 'HCAPLUS' ENTERED AT 13:50:50 ON 14 APR 2004
L1       140589 SEA ABB=ON  PLU=ON  PROTEASE OR PROTEINASE OR PEPTIDEASE OR
                ENDOPEPTIDASE OR ENDOPROTEASE OR ENDOPROTEINASE
L2           39 SEA ABB=ON  PLU=ON  L1 (L) (PYROCOCCUS FURIOSUS)
L3           12 SEA ABB=ON  PLU=ON  L2 (L) (DNA OR CDNA OR POLYNUCLEOTIDE OR
                NUCLEOTIDE OR NUCLEIC ACID)
L4            6 SEA ABB=ON  PLU=ON  L3 AND PD<19970610
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=> d ibib ab 1-6

L4 ANSWER 1 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:779741 HCAPLUS
DOCUMENT NUMBER: 128:125263
TITLE: Homology modeling of two subtilisin-like serine proteases from the hyperthermophilic archaea Pyrococcus furiosus and Thermococcus stetteri
AUTHOR(S): Voorhorst, Wilfried G. B.; Warner, Angela; de Vos, Willem M.; Siezen, Roland J.
CORPORATE SOURCE: Department of Microbiology, Wageningen Agricultural University, Wageningen, NL-6703 CT, Neth.
SOURCE: Protein Engineering (**1997**), 10(8), 905-914
CODEN: PRENE9; ISSN: 0269-2139
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The hyperthermophilic archaeon Pyrococcus furiosus produces an extracellular, glycosylated hyperthermostable subtilisin-like serine protease, termed pyrolysin (Voorhorst, W.G.B., Eggen,R.I.L., Geerling,A.C.M., Platteeuw,C., Siezen,R.J. and de Vos,W.M. (1996) J. Biol. Chemical, 271, 20426-20431). Based on the pyrolysin coding sequence, a pyrolysin-like gene fragment was cloned and characterized from the extreme thermophilic archaeon Thermococcus stetteri. Like pyrolysin, the deduced sequence of this serine protease, designated stetterlysin, contains a catalytic domain with high homol. with other subtilases, allowing homol. modeling starting from known crystal structures. Comparison of the predicted three-dimensional models of the catalytic domain of stetterlysin and pyrolysin with the crystal structure of subtilases from mesophilic and thermophilic origin, i.e. subtilisin BPN' and thermitase, and the homol. model of subtilisin S41 from psychrophilic origin, led to the identification of features that could be related to protein stabilization. Higher thermostability was found to be correlated with an increased number of residues involved in pairs and networks of charge-charge and aromatic-aromatic interactions. These highly thermostable proteases have several extra surface loops and inserts with a relatively high frequency of aromatic residues and Asn residues. The latter are often present in putative N-glycosylation sites. Results from modeling of known substrates in the substrate-binding region support the broad substrate range and the autocatalytic activation previously suggested for pyrolysin.
REFERENCE COUNT: 61 THERE ARE 61 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:728520 HCAPLUS
DOCUMENT NUMBER: 128:71370
TITLE: Methionine aminopeptidase from the hyperthermophilic archaeon Pyrococcus furiosus: molecular cloning and overexpression in Escherichia coli of the gene, and characteristics of the enzyme
AUTHOR(S): Tsunasawa, Susumu; Izu, Yukiko; Miyagi, Masaru; Kato, Ikunoshin
CORPORATE SOURCE: Biotechnology Research Laboratories, Takara Shuzo Co., Ltd., Kusatsu, 525, Japan
SOURCE: Journal of Biochemistry (Tokyo) (**1997**), 122(4), 843-850
CODEN: JOBIAO; ISSN: 0021-924X
PUBLISHER: Japanese Biochemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A gene for a methionine aminopeptidase (MAP; EC 3.4.11.18), which catalyzes the removal of amino-terminal methionine from the growing peptide chain on the ribosome, has been cloned from the hyperthermophilic Archaeon, **Pyrococcus furiosus**, by a novel method

effectively using its cosmid protein library, sequenced and expressed in Escherichia coli. The **DNA** sequence encodes a protein containing 295 amino acid residues with methionine at the N-terminus. From protein analyses of the recombinant protein expressed in E. coli, by using both amino acid sequence anal. from the N-terminus by automated Edman degradation and analyses of mol. masses of the peptides generated by two enzymic cleavages performed independently, digestions with lysyl **endopeptidase** and **Endoproteinase** Asp-N, with ion-spray mass spectrometry, the primary structure of the protein has been elucidated to be completely identical with that deduced from its **DNA** sequence. Comparison of the amino acid sequence of P. furiosus MAP (P.f. MAP) with those of other MAPs from Eukarya and Bacteria showed that the protein has a high degree of sequence homol. in the stretches surrounding the five cobalt-binding residues fully preserved in all of MAPs determined so far, but P.f. MAP belongs to Type II because it has an extra long insertion of about 60 amino acid residues between the fourth and fifth cobalt-binding ligands, similar to MAPs from human and rat, and to Met-AP2 from Saccharomyces cerevisiae, in comparison to Type I MAPs from Bacteria. Therefore, P.f. MAP seems to be rather close to those from Eukarya, although it is distinct in lacking the N-terminal extension of about 90-105 residues universally found in MAPs from Eukarya. These findings suggest that P.f. MAP is evolutionally located at the Eukarya-Bacteria boundary. The enzyme expressed in E. coli exhibits a considerable thermostability, with a half-life of approx. 4.5 h at 90°C and an optimum temperature of around 90°C.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 3 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1996:528382 HCAPLUS

DOCUMENT NUMBER: 125:215332

TITLE: Isolation and characterization of the hyperthermostable serine protease, pyrolysin, and its gene from the hyperthermophilic archaeon Pyrococcus furiosus

AUTHOR(S): Voorhorst, Wilfried G. B.; Eggen, Rik I. L.; Geerling, Ans C. M.; Platteeuw, Christ; Siezen, Roland J.; de Vos, Willem M.

CORPORATE SOURCE: Department Microbiology, Wageningen Agricultural University, Wageningen, 6703 CT, Neth.

SOURCE: Journal of Biological Chemistry (**1996**), 271(34), 20426-20431
CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The hyperthermostable serine protease pyrolysin from the hyperthermophilic archaeon Pyrococcus furiosus was purified from membrane fractions. Two proteolytically active fractions were obtained, designated high (HMW) and low (LMW) mol. weight pyrolysin, that showed immunol. cross-reaction and identical NH2-terminal sequences in which the third residue could be glycosylated. The HMW pyrolysin showwed a subunit mass of 150 kDa after acid denaturation. Incubation of HMW pyrolysin at 95° resulted in the formation of LMW pyrolysin, probably as a consequence of COOH-terminal autoproteolysis. The 4194-base pair pls gene encoding pyrolysin was isolated and characterized, and its transcription initiation site was identified. The deduced pyrolysin sequence indicated a prepro-enzyme organization, with a 1249-residue mature protein composed of an NH2-terminal catalytic domain with considerable homol. to subtilisin-like serine proteases and a COOH-terminal domain that contained most of the 32 possible N-glycosylation sites. The archaeal pyrolysin showed highest homol. with eucaryal tripeptidyl peptidases II on the amino acid level but a different cleavage specificity as shown by its endopeptidase activity

toward caseins, casein fragments including αS1-casein, and synthetic peptides.

L4 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:278327 HCAPLUS
DOCUMENT NUMBER: 125:2522
TITLE: Sequence, expression in Escherichia coli, and analysis of the gene encoding a novel intracellular protease (PfpI) from the hyperthermophilic archaeon Pyrococcus furiosus
AUTHOR(S): Halio, Sheryl B.; Blumentals, Ilse I.; Short, Stephen A.; Merrill, Barbara M.; Kelly, Robert M.
CORPORATE SOURCE: Dep. Chem. Eng., North Carolina State Univ., Raleigh, NC, 27695-7905, USA
SOURCE: Journal of Bacteriology (**1996**), 178(9), 2605-2612
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A previously identified intracellular proteolytic activity in the hyperthermophilic archaeon **Pyrococcus furiosus** (I. I. Blumentals, A. S. Robinson, and R. M. Kelly, Appl. Environ. Microbiol. 56:1992-1998, 1990) was found to be homomultimer consisting of 18.8-kDa subunits. Dissociation of this native P. furiosus **protease** I (PfpI) into a single subunit was seen by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) but only after trichloroacetic acid precipitation; heating to 95°C in the presence of 2% SDS and 80 mM dithiothreitol did not dissociate the protein. The gene (pfpI) coding for this **protease** was located in genomic digests by Southern blotting with probes derived from the N-terminal amino acid sequence. PfpI was cloned, sequenced, and expressed in active form in Escherichia coli as a fusion protein with a histidine tag. The recombinant **protease** from E. coli showed maximum proteolytic activity at 95°C, and its half-life was 19 min at this temperature This level of stability was significantly below that previously reported for the enzyme purified by electroelution of a 66-kDa band from SDS-PAGE after extended incubation of cell exts. at 98°C in 1% SDS (>30 h). The pfpI gene codes for a polypeptide of 166 amino acid residues lacking any conserved **protease** motifs; no **protease** activity was detected for the 18.8-kDa PfpI subunit (native or recombinant) by substrate gel assay. Although an immunol. relationship of this **protease** to the eukaryotic proteasome has been seen previously, searches of the available databases identified only two similar amino acid sequences: an open reading frame of unknown function from Staphylococcus aureus NCTC 8325 (171 amino acid residues, 18.6 kDa, 41% identity) and an open reading frame also of unknown function in E. coli (172 amino acid residues, 18.8 kDa , 47% identity). Primer extension expts. with P. furiosus total RNA defined the 5' end of the transcript. There are only 10 **nucleotides** upstream of the start of translation; therefore, it is unlikely that there are any pre- or pro-regions associated with PfpI which could have been used for targeting or assembly of this **protease**. Although PfpI activity appears to be the dominant proteolytic activity in P. furiosus cell exts., the physiol. function of PfpI is unclear.

L4 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1996:113482 HCAPLUS
DOCUMENT NUMBER: 124:169387
TITLE: Cloning of gene for hyperthermostable proteinase of Pyrococcus furiosus
INVENTOR(S): Mitta, Masanori; Yamamoto, Katsuhiko; Morishita, Mio; Asada, Kiyozo; Tsunasawa, Susumu; Kato, Ikunoshin
PATENT ASSIGNEE(S): Takara Shuzo Co., Ltd., Japan

SOURCE: PCT Int. Appl., 85 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
WO 9534645	A1	19951221	WO 1995-JP1095	19950605	<--
W: CN, JP, US					
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE					
EP 776971	A1	19970604	EP 1995-920251	19950605	<--
EP 776971	B1	20011205			
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE					
CN 1154717	A	19970716	CN 1995-194487	19950605	
CN 1086737	B	20020626			
AT 210181	E	20011215	AT 1995-920251	19950605	
US 5756339	A	19980526	US 1996-750532	19961213	

PRIORITY APPLN. INFO.: JP 1994-130236 A 19940613
JP 1994-173912 A 19940726
WO 1995-JP1095 W 19950605

AB A hyperthermostable proteinase gene originating in Pyrococcus furiosus was isolated and its amino acid sequence deduced. The gene can be used for the production of the hyperthermostable proteinase.

L4 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1995:364954 HCAPLUS
DOCUMENT NUMBER: 122:257622
TITLE: A gene from the hyperthermophile Pyrococcus furiosus whose deduced product is homologous to members of the prolyl oligopeptidase family of proteases
AUTHOR(S): Robinson, Kelly A.; Bartley, Duane A.; Robb, Frank T.; Schreier, Harold J.
CORPORATE SOURCE: Center of Marine Biotechnology, University of Maryland Biotechnology Institute, Baltimore, MD, 21202, USA
SOURCE: Gene (**1995**), 152(1), 103-6
CODEN: GENED6; ISSN: 0378-1119
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The mlr-2 gene from the hyperthermophilic archaeum **Pyrococcus furiosus** was identified from a family of clones whose expression was influenced by the presence of maltose in the medium. The sequence of 2100 bp of **DNA** containing mlr-2 and its flanking regions revealed a 616-amino-acid (71 kDa) open reading frame (ORF). The ORF's initiation codon appeared 10 nt into the mlr-2 message and was not preceded by any apparent ribosome-binding site. The deduced product shared homol. with prolyl **endopeptidases** from both eukaryotic and eubacterial sources (52-57% similarity, 30-37% identity) and signature domains containing the Ser-Asp-His triad, which is characteristic of this family of **proteases**, were present. Northern blot expts. revealed the presence of an .apprx.2.0-kb transcript in P. furiosus exts., corresponding in length to that expected from mlr-2 expression. Initiation of transcription occurred 23 bp downstream from a putative BoxA promoter element.